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Neoseiulus paspalivorus, a predator from coconut, as a candidate for controlling dry bulb mites infesting stored tulip bulbs

Izabela Lesna · Fernando R. da Silva · Yukie Sato · Maurice W. Sabelis · Suzanne T. E. Lommen

Abstract The dry bulb mite, *Aceria tulipae*, is the most important pest of stored tulip bulbs in The Netherlands. This tiny, eriophyoid mite hides in the narrow space between scales in the interior of the bulb. To achieve biological control of this hidden pest, candidate predators small enough to move in between the bulb scales are required. Earlier experiments have shown this potential for the phytoseiid mite, *Neoseiulus cucumeris*, but only after the bulbs were exposed to ethylene, a plant hormone that causes a slight increase in the distance between tulip bulb scales, just sufficient to allow this predator to reach the interior part of the bulb. Applying ethylene, however, is not an option in practice because it causes malformation of tulip flowers. In fact, to prevent this cosmetic damage, bulb growers ventilate rooms where tulip bulbs are stored, thereby removing ethylene produced by the bulbs (e.g. in response to mite or fungus infestation). Recently, studies on the role of predatory mites in controlling another eriophyoid mite on coconuts led to the discovery of

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an exceptionally small phytoseiid mite, *Neoseiulus paspalivorus*. This predator is able to move under the perianth of coconuts where coconut mites feed on meristematic tissue of the fruit. This discovery prompted us to test *N. paspalivorus* for its ability to control *A. tulipae* on tulip bulbs under storage conditions (ventilated rooms with bulbs in open boxes; 23 °C; storage period June–October). Using destructive sampling we monitored predator and prey populations in two series of replicated experiments, one at a high initial level of dry bulb mite infestation, late in the storage period, and another at a low initial dry bulb mite infestation, halfway the storage period. The first and the second series involved treatment with *N. paspalivorus* and a control experiment, but the second series had an additional treatment in which the predator *N. cucumeris* was released. Taking the two series of experiments together we found that *N. paspalivorus* controlled the populations of dry bulb mites both on the outer scale of the bulbs as well as in the interior part of the bulbs, whereas *N. cucumeris* significantly reduced the population of dry bulb mites on the outer scale, but not in the interior part of the bulb. Moreover, *N. paspalivorus* was found predominantly inside the bulb, whereas *N. cucumeris* was only found on the outer scale, thereby confirming our hypothesis that the small size of *N. paspalivorus* facilitates access to the interior of the bulbs. We argue that *N. paspalivorus* is a promising candidate for the biological control of dry bulb mites on tulip bulbs under storage conditions in the Netherlands.

Keywords Biological control · Tulip bulbs · Dry bulb mites · Eriophyidae · Predatory mites · Phytoseiidae · Predator–prey interaction · Body size · Prey refuge · Ethylene · Herbivore-induced plant response · New association

Introduction

Eriophyoid mites are among the smallest arthropods on Earth (Lindquist et al. 1996). Their worm-like body has a cross-section diameter of c. 50 μm, much smaller than that of phytoseiid mites, their most significant predators (Sabelis 1996). The minute size of eriophyoid mites is the key to their ecological success, enabling them to reach places small enough to be free of predators (Sabelis 1996; Sabelis and Bruin 1996). Moreover, it allows them to develop a plant-parasitic lifestyle that is quite different from other herbivorous arthropods (Lindquist et al. 1996). Many eriophyoids live in plant galls they induce, but the one of interest in this article have a vagrant lifestyle, frequently changing feeding sites that vary in food quality and in the degree of protection against predators. In agricultural crops, such mites may reach pest status when natural enemies are absent. Chemical control is often ineffective because most eriophyoid mites feed under protective structures of the plant. Hence, biological control with natural enemies, such as predatory mites or acaropathogens, may be the only solution (Sabelis et al. 2007, 2008).

In this article, we consider the eriophyid *Aceria tulipae* Keifer on tulip bulbs. This so-called dry bulb mite is the most important pest in the tulip bulb industry in the Netherlands. After harvest, tulip bulbs are stored for several months in rooms at temperatures (≥23 °C) that promote population growth of the dry bulb mites. In this period, they receive pesticide treatments that reduce the pest and remove virtually all predators associated with bulbs in the field. Thus, if pesticides are not applied well enough, favourable temperatures and absence of predators cause the dry bulb mites to reach pest status whereby leaves, flowers,

shoots and roots are malformed or do not even emerge from the bulb (Conijn et al. 1996; Van Aartrijk 2000; Aratchige et al. 2004; Lesna et al. 2005). Due to this cosmetic damage and the presumed role of dry bulb mites as a vector of Tulip Virus X (Asjes and Blom-Barnhoorn 1998, de Kock et al. 2011, Lommen et al. 2012a) this pest is treated as a quarantine organism in several important export-countries. Hence, a zero pest tolerance is the general norm.

Chemical control is currently widely applied by tulip growers, as well as physical treatments by organic farmers. Nevertheless, the dry bulb mite causes severe economic losses each year. A key problem in effectively controlling dry bulb mites is that its minute size enables a hidden life in the tulip bulb. Like onions, a tulip bulb consists of multiple layers of concentric scales of increasing diameter, surrounding the center where the stem, leaves and flower develop. The dry bulb mite is so tiny (cross-section diameter = c. 0.06 mm) that it can access the interior of the bulb by moving between its scales (Lesna et al. 2005). Biological control may be the only solution (Sabelis et al. 2007, 2008), provided that the control agents can access the interior of the bulbs as well.

Phytoseiid mites are the most significant predators of eriophyoid mites (Sabelis 1996), and several species are known to feed and reproduce on dry bulb mites in the laboratory (Lesna et al. 2005). In contrast to dry bulb mites, predatory mites are too big to move between the scales of the bulb initially (Lesna et al. 2005). However, feeding by dry bulb mites gradually induces the bulbs to modify their internal structure. The resulting changes in distance between bulb scales (from 0.1 to 0.2 mm) are microscopic, yet allow the phytoseiid predator *Neoseiulus cucumeris* (Oudemans) (with a somal width of 0.2 mm and a somal height of 0.12 mm) to enter the interior bulb space (via the so-called "nose" of the bulb). The consequence of this plant 'behaviour' is dramatic: predators eliminate most eriophyid mites from the inside of the bulb, whereas the bulb would otherwise be eaten from within. The crucial changes in bulb morphology enabling predator access are controlled by ethylene, a plant hormone released (among others) upon herbivore attack (Lesna et al. 2005). This plant hormone simultaneously induces the release of plant volatiles that attract predatory mites (Aratchige et al. 2004; Aratchige 2007). Changes in bulb attractiveness and accessibility were demonstrated by combining chemical analysis (Aratchige 2007), olfactometry (Aratchige et al. 2004) and experiments on predator–prey dynamics in which the effect of ethylene was either promoted or blocked by 1-methyl-cyclopropene, a chemical occupying ethylene receptor sites (Lesna et al. manuscript in preparation). In climate cabinets ventilated to remove ethylene, short, 2-weekly exposure of bulbs to ethylene had major effects on the ability of the predatory mite *N. cucumeris* to control *A. tulipae* in the interior of the bulb when compared to identical exposure to ethylene blockers or ambient air. Exposure to ethylene and its blockers had no direct effect on dry bulb mites and predatory mites and at the concentrations offered ethylene exposure had similar effects on the morphology of healthy bulbs as exposure to (ethylene and other odours from) infested tulip bulbs. Taken together, these results explain why earlier experiments yielded successful biological control of dry bulb mites by *N. cucumeris* on tulip bulbs in closed cardboard boxes with consequently higher ethylene concentrations, but were not successful in open trays in ventilated climate rooms (Lesna et al. 2005; Sabelis et al. 2007, 2008, 2012).

However, ethylene exposure of tulip bulbs is not an option for the flower bulb industry. In fact, in practice ethylene is continuously diluted and led away from the storage rooms by ventilation, because of its adverse effects on flower development (de Munk 1973, Kamerbeek and de Munk 1976, Gude and Dijkema 2005) and increased risks of bud necrosis (Lommen et al. 2012b). Hence, there is a need to find predators that do not only

feed on the dry bulb mite, but are also small enough to enter the inside of the bulb under storage conditions in practice. Such a small phytoseiid predator has recently been found on coconuts: *Neoseiulus paspalivorus* (De Leon) (Lawson-Balagbo et al. 2008; Negloh et al. 2011). The female soma of *N. paspalivorus* has a cross-section diameter that is only about half of that of *N. cucumeris* (da Silva, personal observation). The predator lives mainly under the perianth of the coconut and feeds on another eriophyid, the coconut mite, *Aceria guerreronis* Keifer (Lawson-Balagbo et al. 2008). Because the climatic conditions in coconut growing areas and the microhabitat under the coconut perianth are not too different from those in tulip bulbs under storage conditions, we decided to do biocontrol tests with this predator in ventilated rooms at 23 °C. These experiments were done with the aim to test (1) whether the predator is able to control dry bulb mites on and in tulip bulbs, and (2) whether its small size facilitates access to the interior of tulip bulbs more than the size of *N. cucumeris*. Two series of experiments were carried out, one with initially high levels of infestation by dry bulb mites and another with initially low levels. Whereas the first series may easily lead to such high pest densities that not only predation, but also intraspecific food competition drive pest mortality, the second series decreases the role of intraspecific competition and therefore puts the focus more on the role of predation. Moreover, high densities of dry bulb mites may also lead to (undesirable) higher ethylene levels despite ventilation. However, note that in the second series the role of ethylene in making the bulb more accessible was minimized because apart from removal by ventilation the herbivore-induced ethylene production was low as a consequence of the low density of dry bulb mites.

Materials and methods

Mite origin and rearing

The predatory mite, *N. paspalivorus*, was collected in 2011 near Recife, Brazil, from coconuts infested by *A. guerreronis*. After shipment to the University of Amsterdam, it was reared on the eriophyid *Aculops lycopersici* (Massee) on tomato leaves in a climate room (25–27 °C, 65 % RH). This eriophyid was the best available alternative prey to rearing on *A. guerreronis* on cocunuts, which was not feasible in Amsterdam.

The predatory mite, *N. cucumeris*, was reared on fungivorous acarid mites and directly provided by Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands) in numbers sufficient for our experiments in 2012.

In September 2011 dry bulb mites (*A. tulipae*) were collected from recently harvested and infested tulip bulbs (cv "Leen van der Mark") in Lisse, and subsequently reared on organic, unsprayed, garlic bulbs in the laboratory (25 °C, 65 % RH) at the University of Amsterdam.

Plant material

We used two cultivars of tulip bulbs, known to be susceptible to dry bulb mites. For the first experiment, we used untreated, mite-free bulbs cv "Leen van der Mark", harvested in New Zealand in the second half of January 2012 and then shipped to The Netherlands. They were stored in open trays of 500 bulbs in ventilated climate boxes (17–23 °C, 70 %RH) at the research station in Lisse.

For the second experiment we used untreated, mite-free bulbs cv "Yokohama", harvested in July 2012 in The Netherlands, and stored in open trays of 500 bulbs in a ventilated climate-room (20 °C, RH not regulated) in Lisse.

Biocontrol experiments

In 2012, two different biological control experiments were carried out, the details of which are summarized in Table 1. Infestation of tulip bulbs was established in separate climate boxes (23 °C; 70 % RH). After transfer of the tulip bulbs, we systematically distributed c. 50 garlic cloves heavily infested with dry bulb mites among c. 500 (initially mite-free) bulbs. The garlic cloves were regularly redistributed between the bulbs, and new cloves were added until the desired infection rate was obtained to start the experiments.

Experiments were carried out in a storage room at a constant temperature of 23 °C, as commonly done in practice during tulip bulb storage. The ventilation rate in the storage room was 2–3 times lower than in practice, but the density of tulip bulbs in the room (and hence the emission of ethylene) was also much lower than in practice, making strong ventilation less necessary. Each replicate experiment was carried out in a plastic bucket filled with bulbs infested with dry bulb mites. To prevent mite migration between replicates in the experiments, buckets were provided with double-sided sticky tape just below the rim. In addition, each bucket was placed in a tray filled with water and detergent decreasing water tension. The trays were placed next to each other at a distance of at least 5 cm, whereas the buckets in these trays were at a distance of 0.5 m.

The buckets were randomly assigned to treatments (treatments with different predator species and control treatments without predators). Maximally 24 h before application of the treatments, predatory mites were collected from their laboratory cultures using a pipette connected to an aspirator. Per pipette, about 50 adult females were collected. Pipettes were then closed with Parafilm on the narrow opening, and with fine mesh gauze (for ventilation) at the other end. The fine mesh gauze was fixed into the pipette with a small piece of plastic tube. Just before release, the pipettes were held vertically with the gauze-covered opening down and they were gently tapped to make the predators fall down on the gauze. Then, the pipette was disassembled and its parts (gauze, pipette, piece of tube) were immediately deposited on the infested tulip bulbs in a bucket.

Starting from the date of the predator releases, each week four bulbs were collected per bucket (replicate unit) and they were inspected under a binocular microscope for the number of dry bulb mites (only mobile stages) and predatory mites (all stages, including eggs) on the outer scale (directly under the dry bulb skin) and separately in the interior of the bulbs. For the latter, the bulb was first cut in half and then the scales were sequentially removed and inspected under the microscope. Because predatory mites are much more agile than dry bulb mites, we were not able to separate predator densities at the outer scale and in the interior of the bulb. Hence, we only determined total predator density per bulb. All data from each of the four bulbs (dry bulb mite density per bulb inside, outside or overall; overall predator density per bulb) were summed and divided by the number of bulbs to obtain a mean number of mites per bulb in the sample. Only these mean numbers per bulb per sample were included in the data set that was subjected to statistical analysis.

Experiment 1: high infestation, late in the storage period

The first experiment was carried out with tulip bulbs cv "Leen van der Mark" from New Zealand. They were harvested in January 2012, transported to Lisse (The Netherlands) and

Table 1 Set-up of the biological control experiments with *N. paspalivorus* and *N. cucumeris*

Experiment	Tulip bulbs			Timing		Set-up					
	Origin	cv	Harvest	Start	Duration (weeks)	No. bulbs/ replicate	Initial no. *Aceria*/bulb	No. replicates/treatment			Released no. predators/ bulb
								C	Nc	Np	
1. high infestation, late in storage	New Zealand	Leen van der Mark	Late January	Early June	3	34	1,590	3	–	3	5.9
2. low infestation, halfway storage	Netherlands	Yokohama	Early July	Mid Sept	5	80	16	4	4	4	2.5

C control, *Nc Neoseiulus cucumeris*, *Np Neoseiulus paspalivorus*

kept in storage for 5 months (thus, late in the storage period) before use in the experiment. On June 4, 2012 the experiment started and then the level of dry bulb mite infestation of the bulbs was high (on average 1590 dry bulb mites per bulb). To test whether predatory mites can suppress such high numbers of dry bulb mites, we released either no predators (control treatment) or only *N. paspalivorus* (treatment). For each of these two treatments three replicates were performed involving 3 buckets, with 34 bulbs each. Just after release, the predator density was on average 5.9 per bulb. The experiment lasted 3 weeks since predator release.

Experiment 2: low infestation halfway the storage period

The second experiment was carried out with tulip bulbs cv "Yokohama" from The Netherlands. They were harvested in early July 2012 and kept in storage for 3 months (thus, halfway the storage period) before use in the experiment. On September 17, 2012 the experiment started and then the level of dry bulb mite infestation of the bulbs was low (on average 16 dry bulb mites per bulb).

To test whether predatory mites can keep low numbers of dry bulb mites down, we released no predators (control treatment), only *N. paspalivorus* or only *N. cucumeris* (treatments). For each of these three treatments four replicates were performed involving four buckets, with 80 bulbs each. Just after release, the predator density was on average 2.5 per bulb. The experiment lasted 5 weeks since predator release.

Statistical analysis

To analyze the longitudinal data series of dry bulb mite densities (actually means per bulb from 4 bulbs, as explained above), we used a multivariate generalized linear mixed model (glmm) with Markov chain Monte Carlo (MCMC) estimation (MCMCglmm; Hadfield 2010), which is available in the statistical package R version 2.14.2 (R Core Team 2011). Densities of dry bulb mites in the interior and on the exterior part of the bulbs were analysed separately for either of the two experiments. We used the log-transformed mean number of dry bulb mites per bulb per replicate as the response variable. To compare the changes in dry bulb mite density over weeks between treatments, models were constructed with treatments (control, *N. paspalivorus* and, for experiment 2, additionally *N. cucumeris*) and time (weeks after introduction of dry bulb mites) as well as their interactions as the fixed factors, and with replicate within each week as a random factor. In the first series of experiments, log-transformed dry bulb mite density and weeks showed a quadratic relation in the control treatment. Therefore, we added the squared weeks and its interaction with treatment as extra fixed effects for this analysis. We used a Gaussian error distribution for the model and an inverse gamma prior ($V = 1$, $nu = 0.002$) in the G-matrix (random effects) and the R-matrix (error structure). We applied default settings for other options in MCMCglmm (starting value, etc.). We ran each analysis for 150,000 iterations with a burn-in of 50,000 iterations and a thinning interval of 10. This generated 10,000 samples from each chain. We then first checked whether autocorrelations between successive stored iterations are <0.1, and checked the model by plotting the distribution of the coefficients and the residual variances of the sampled posterior. Terms were considered statistically significant when 95 % confidence intervals did not overlap and P-MCMC < 0.05.

To analyze the longitudinal data on the predator densities in the second experiment, we applied again an MCMCglmm. We used the log-transformed values of the mean number of predators + 1, per bulb per replicate. To compare the changes in the predator densities

over weeks between *N. paspalivorus* and *N. cucumeris*, a model was constructed with predator species (*N. paspalivorus*, *N. cucumeris*) and time (weeks after predator introduction) as well as their interactions as fixed factors, and with replicate within each week as a random factor. We then analysed these data in the same way using the same parameters as applied in the analysis of dry bulb mite densities.

Results

General impact of predators

There was a significant difference in the change of total densities of dry bulb mites over time between the treatment with *N. paspalivorus* and the control in either of the two experiments (Tables 2, 3; Fig. 1, 2). This predator was able to reduce pest densities both inside and on the outer scale of the bulbs. The second experiment, including treatments with *N. cucumeris*, showed that *N. cucumeris* also suppressed the density of dry bulb mites on the outer scale (Table 3b; Fig. 2). However, *N. cucumeris* did not significantly suppress the density of dry bulb mites inside bulbs (Table 3a; Fig. 2).

Experiment 1: high infestation late in the storage period

In the first experiment, initial total densities of dry bulb mites averaged 1590 per bulb. After three weeks, the average had decreased to 170 in control treatments and to 37 in treatments with *N. paspalivorus* (Fig. 1a). Regardless of treatment, most dry bulb mites were found on the outer scale of the bulbs (Fig. 1b vs. 1c). However, the development of

Table 2 Results of MCMCglmm for the *Aceria tulipae* densities (a) inside bulbs and (b) outside bulbs in experiment 1 (initially high infestation, late in storage period, predator *Neoseiulus paspalivorus*)

Explanatory variable	Posterior mean (CI)	*P*-MCMC[a]
(a)		
Intercept	5.72 (2.52 to 9.18)	**0.002**
Treatment (*N. paspalivorus*)	3.20 (−1.49 to 7.85)	0.15
Week	1.09 (−1.85 to 4.13)	0.46
I (Week^2)	−0.38 (−0.97 to 0.20)	0.20
Treatment (*N. paspalivorus*)*Week	−3.53 (−7.76 to 0.73)	0.092
Treatment (*N. paspalivorus*)*I (Week^2)	0.63 (−0.19 to 1.48)	0.12
(b)		
Intercept	1.43 (−2.06 to 4.89)	0.40
Treatment (*N. paspalivorus*)	4.24 (−0.62 to 9.07)	0.080
Week	7.20 (3.96 to 10.42)	**<0.001**
I (Week^2)	−1.80 (−2.47 to −1.22)	**<0.001**
Treatment (*N. paspalivorus*)*Week	−5.33 (−9.96 to −0.94)	**0.025**
Treatment (*N. paspalivorus*)*I (Week^2)	1.10 (0.24 to 2.00)	**0.018**

CI = 95 % confidence interval

[a] The probability *P* that the parameter value is more than 0 or less than 0 calculated in MCMCglmm. Bold type represents $P < 0.05$

Table 3 Results of MCMCglmm for the *Aceria tulipae* densities (a) inside bulbs and (b) outside bulbs in experiment 2 (initially low infestation, halfway the storage period, predators *Neoseiulus cucumeris* or *N. paspalivorus*)

Explanatory variable	Posterior mean (CI)	*P*-MCMC[a]
(a)		
Intercept	1.37 (0.70 to 2.05)	**<0.001**
Treatment (*N. cucumeris*)	0.16 (−0.81 to 1.11)	0.73
Treatment (*N. paspalivorus*)	0.11 (−0.87 to 1.04)	0.82
Week	0.75 (0.57 to 0.95)	**<0.001**
Treatment (*N. cucumeris*)*Week	−0.08 (−0.34 to 0.19)	0.54
Treatment (*N. paspalivorus*)*Week	−0.66 (−0.93 to −0.39)	**<0.001**
(b)		
Intercept	1.29 (0.57 to 2.00)	**<0.001**
Treatment (*N. cucumeris*)	−0.31 (−1.31 to 0.69)	0.53
Treatment (*N. paspalivorus*)	−0.06 (−1.08 to 0.95)	0.92
Day	1.06 (0.87 to 1.25)	**<0.001**
Treatment (*N. cucumeris*)*Day	−0.53 (−0.79 to −0.27)	**<0.001**
Treatment (*N. paspalivorus*)*Day	−0.77 (−1.04 to −0.51)	**<0.001**

CI = 95 % confidence interval

[a] The probability *P* that the parameter value is more than 0 or less than 0 calculated in MCMCglmm. Bold type represents $P < 0.05$

the dry bulb mite populations differed dramatically between the treatments with *N. paspalivorus* and the control (Fig. 1a; Table 2). Whereas the number of dry bulb mites first increased in the control treatments, they did not in the predator treatments. Specifically, the control treatments showed an increase of the total density of dry bulb mites during the first week (1 replicate) or the first 2 weeks (2 replicates), followed by a strong decrease (Fig. 1a). In contrast, the treatment with the predatory mite *N. paspalivorus* led to a substantial decrease of the density of dry bulb mites in the first week (1 replicate) or in the first 2 weeks (2 replicates), but not in the last week(s) (Fig. 1a). This pattern of decrease in dry bulb mite density was manifested equally on the outer scale and in the interior of the bulb (Fig. 1b, c). The differences in change of dry bulb mite densities over time were significant for the outer scale of the bulb (Table 2b), but not in the interior of the bulb (Table 2b).

Experiment 2: low infestation halfway the storage period

In the second experiment, the effects of predator treatments were visible up to the last week of the experiment. The total density of dry bulb mites was initially 16 per bulb on average (7 on the outer scale, 9 inside the bulb). Whereas the control experiments showed exponential growth of the total population of dry bulb mites, treatments with the predatory mite *N. paspalivorus* led to virtually no increase of dry bulb mite densities, and *N. cucumeris* showed an intermediate level (Fig. 2a). The effect of *N. paspalivorus* was highly significant, both on the outer bulb scale ($P < 0.001$, Treatment (*N. paspalivorus*)*Day in Table 3b; Fig. 2b) and in the interior of the bulbs [$P < 0.001$, Treatment (*N. paspalivorus*)*Day in Table 3a; Fig. 2c]. In contrast, treatment with the predatory mite *N. cucumeris* had a significant impact on dry bulb mite density on the outer bulb scale [$P < 0.001$,

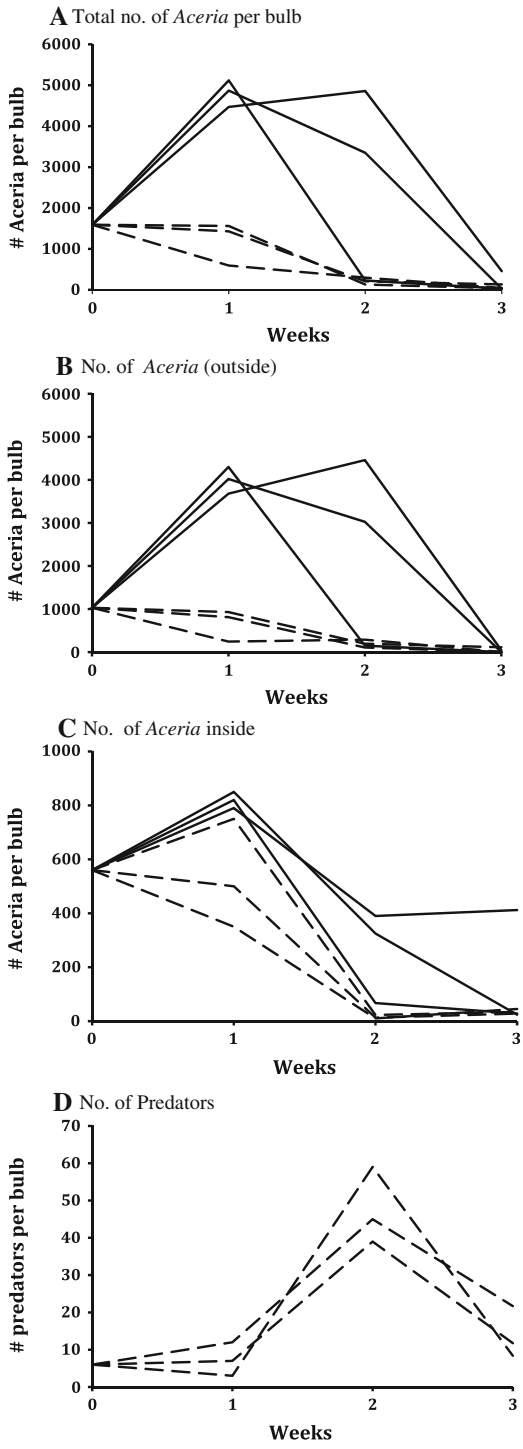

Fig. 1 Temporal dynamics of *Aceria tulipae* (mean number per bulb, i.e. mean of 4 bulbs): on and in the bulb (total numbers) (**a**), then separately for the outer scale (**b**) and in the interior of the bulb (**c**), in the control treatment (*drawn line*) and in the treatment with the predatory mite *Neoseiulus paspalivorus* (*dashed line*). In panel (**d**) the dynamics of the predatory mites (mean total per bulb, i.e. mean of 4 bulbs) is shown (*dashed line*). Results are presented for each of the three replicates separately

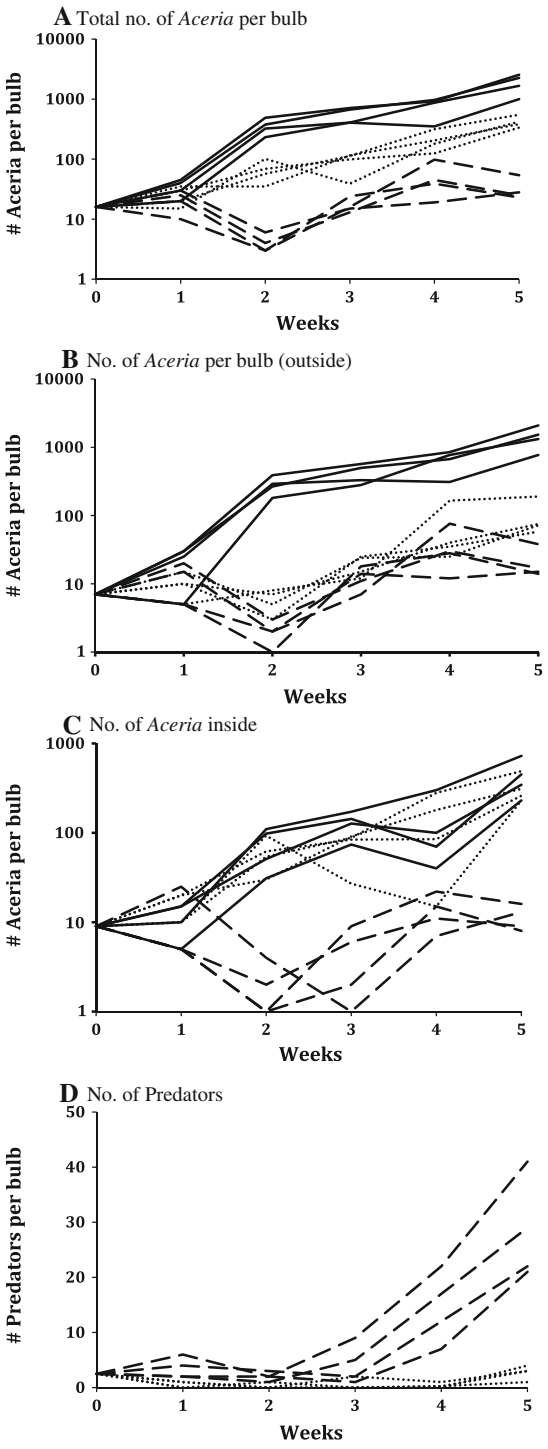

Fig. 2 Temporal dynamics of *Aceria tulipae* (mean number per bulb, i.e. mean of 4 bulbs): on and in the bulb (total numbers) (**a**), then separately for the outer scale (**b**) and in the interior of the bulb (**c**), in the control treatment (*drawn line*), in the treatment with the predatory mite *Neoseiulus paspalivorus* (*dashed line*) and in the treatment with the predatory mite, *N. cucumeris* (*dotted line*). In panel (**d**) the dynamics of the predatory mites (mean total per bulb, i.e. mean of 4 bulbs) is shown (*N. paspalivorus*, *dashed line*; *N. cucumeris*, *dotted line*). Results are presented for each of the four replicates separately

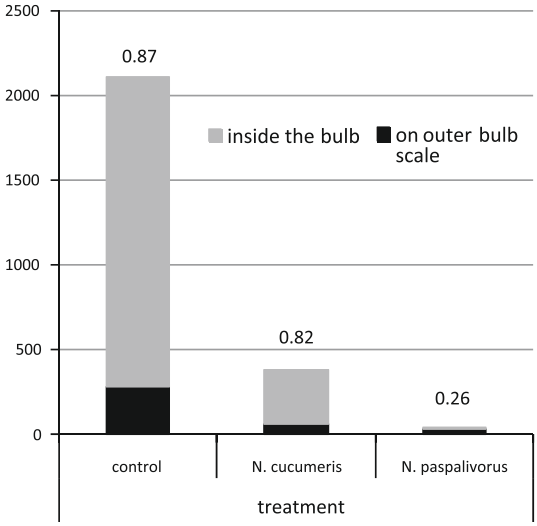

Fig. 3 Spatial distribution of *Aceria tulipae* on the bulbs. Bars represent mean numbers of *A. tulipae* per bulb for each treatment on the outer scale of the bulbs (*black*) and inside the bulbs (*grey*), at the end (after 5 weeks) of experiment 2 with initial low levels of infestation (16 *A. tulipae*/bulb). Numbers above bars indicate the fraction of *A. tulipae* found inside the bulbs

Treatment (*N. cucumeris*)*Day in Table 3b; Fig. 2b], but not in the interior of the bulb. Treatment with *N. cucumeris* could not curb exponential increase of dry bulb mites inside the bulb. It gave rise to a lower but non-significantly different growth rate than in the control experiments [$P = 0.54$, Treatment (*N. cucumeris*)*Day in Table 3a; Fig. 2c]. As a result, not only the numbers of dry bulb mites differed between treatments at the end of the experiment, but also their spatial distribution over the outer and inner bulb (Fig. 3). In control treatments, 87 % of dry bulb mites were found inside the bulb. The predator *N. cucumeris* could not prevent the total dry bulb mite population from growing, but reduced the total numbers of dry bulb mites compared to the control, and 82 % of these remaining dry bulb mites was found inside the bulb. In contrast, *N. paspalivorus* reduced total numbers of dry bulb mites dramatically when compared to the control, and reversed the pattern of spatial distribution: only 26 % of the remaining mites was found inside the bulbs at the end of the experiment (Fig. 3).

Predator populations

Release of *N. paspalivorus* led to substantial population growth of this predator in each of the two experiments (Fig. 1d and 2d; peaking on average at 48 and 28 predators respectively), but release of *N. cucumeris* (only carried out in the second experiment), led to a rather marginal increase in population size (Fig. 2d; peaking on average at 3 predators). This difference in population growth of the two predators over time was highly significant ($P < 0.01$, Treatment (*N. paspalivorus*)*Week in Table 4). During sampling, we observed *N. paspalivorus* on the outer scale, as well as in the interior of the bulbs in the first experiment but mainly in the interior of the bulbs in the second experiment. However, *N. cucumeris* was never found inside the bulbs.

Discussion

We conclude that the predatory mite *N. paspalivorus* can control dry bulb mites on tulip bulbs in open buckets in ventilated rooms, representing conditions close (but not equal) to

Table 4 Results of MCMCglmm for the total predator densities in the in experiment 2 (initially low infestation, halfway the storage period, predator *Neoseiulus paspalivorus*)

Explanatory variable	Posterior mean (CI)	*P*-MCMC[a]
Intercept	0.60 (0.09 to 1.19)	**0.031**
Treatment (*N. paspalivorus*)	−0.18 (−0.98 to 0.55)	0.65
Week	0.0001 (−0.15 to 0.15)	0.99
Treatment (*N. paspalivorus*)*Week	0.41 (0.19 to 0.63)	**0.001**

CI = 95 % confidence interval

[a] The probability *P* that the parameter value is more than 0 or less than 0 calculated in MCMCglmm. Bold type represents $P < 0.05$

those of tulip bulb storage in practice (Figs. 1a, 2a). This predator was much more effective in controlling this pest than *N. cucumeris* (Figs. 2a, 3a). Moreover, it should be stressed that ventilation removes most of the ethylene so that under the conditions of our experiments there were no negative effects of flower malformation to be expected.

Predator-to-prey size and interior bulb structure as a key to biocontrol success

We argue that the success of *N. paspalivorus* is explained by its small size. We observed that this predator managed to move into the interior part of the bulb, probably due to its small size and elongated body shape, and possibly also by behavioural adaptations (F. da Silva, personal observations; experiments in progress). In contrast, the larger *N. cucumeris* was never found in the interior part of the tulip bulbs, which is likely to explain its failure to control dry bulb mites inside the bulbs (Fig. 2c).

However, accessibility of the bulb interior is not only determined by predator size, but also by environmental conditions altering the bulb structure. During storage in practice temperatures decrease gradually (from 23 to 17 °C) and bulbs desiccate very slowly, whereas in our storage experiments the bulbs were kept at 23 °C and had already been stored at 20 °C for more than 4 months, likely resulting in faster desiccation and better access. Ethylene levels might also have differed in our experiments from those in storage rooms in practice, and consequently affected accessibility, but it is hard to say in what way they differed. On the one hand, they could have been higher in our experiments, and consequently facilitated access, because our ventilation rates were 2–3 times lower than in practice, and initial dry bulb mite densities were high in the first experiment (Lesna et al. 2005). On the other hand, the number and density of bulbs in our experiments was nothing compared to what is usual in practice, and the density of dry bulb mites was very low in the second experiment. How all these factors together determine accessibility of the bulbs in our experiments compared to that in practice is difficult to evaluate. Thus, whether *N. paspalivorus* will be equally effective in practice as in our experiments, still requires scrutiny by means of further biocontrol tests.

Predation and intra-specific competition as pest mortality factors

Pest mortality is not only due to predation, but possibly also to intraspecific competition for food and a decreasing quality of bulbs as a food source. Especially, the control treatment of experiment 1 shows evidence for the latter. This is because the initial density of dry bulb mites was orders of magnitude higher than in experiment 2: on average 1590 per bulb in experiment 1 versus 16 per bulb in experiment 2 (Figs. 1a, 2a). Moreover, in the control

treatment of experiment 1 the density of dry bulb mites increased during the first 1 or 2 weeks, but decreased during the last 1 or 2 weeks. This pattern is clearly in contrast with the dynamics in the control of experiment 2, where the dry bulb mites showed exponential growth over the full 5 weeks of the experiment (Fig. 2a). Thus, intraspecific competition is more likely to play a role in experiment 1 than in experiment 2.

However, even though density-dependent mortality due to intraspecific competition and bulb quality may play an important role in experiment 1, there is strong evidence that predation acts in concert, also in this experiment. In the first week after predator release the predator-treated replicates showed equal or decreasing total numbers of dry bulb mites, whereas the control treatments showed a threefold increase in pest numbers in the same period. In the next 2 weeks mortality factors other than predation are likely to be more important and they may well reflect intraspecific competition for food, as a result of increasing pest numbers and decreasing bulb quality. This pattern is commonly observed in practice towards the end of the storage period.

Experiment 2 clearly indicates that *N. paspalivorus* has potential to control dry bulb mites relatively early in the bulb storage period, when densities of dry bulb mites are still low. We argue that the continued population decline of dry bulb mites in the predator treatments can be attributed largely to predation and not to intra-specific competition for food, because the control treatments show exponential increase of dry bulb mites throughout the experiment (Fig. 2a).

Why no spontaneous emergence of predatory mites in storage rooms?

Because *N. paspalivorus* originates from coconuts in Brazil and is used in our experiments in the Netherlands to control eriophyid mites on tulip bulbs, a completely different plant, this predator and prey surely represent a novel association. This prompts the question whether there are no suitable predatory mites in the Netherlands that spontaneously emerge under storage conditions and control dry bulb mites. The climatic conditions under storage conditions are not only ideal for the dry bulb mites, but also for predatory mites. This lack of predators in practice is largely due to fumigation of bulbs with pirimiphos-methyl (Actellic-50®) during bulb storage. This pesticide is lethal for most predatory mites, as well as for dry bulb mites. Just after harvest from the field, the scales of the tulip bulbs are so tightly fitting together that only dry bulb mites, but not predatory mites, might have a chance to hide inside the bulb. Fumigation will kill most mites on the exterior part of the bulb, but less so in the bulb interior. Thus, dry bulb mites have a relatively higher chance of escaping from the effect of fumigation and, if so, they enter enemy-free space, once they arrive with the bulbs in the storage room.

Thus, to detect potential predators for dry bulb mite control in The Netherlands, predators have to be collected from the field or from stored bulbs not receiving a pre-treatment with pesticides. This has been done in bulb stocks stored in the laboratory in Amsterdam and at the Flower bulb Research Centre in Lisse (currently known as PPO) (Lesna et al. 2005). The predatory mite species recorded were *Cheyletus eruditus* (Schrank), *Lasioseius fimetorum* Karg, *Blattisocius* spp., *N. cucumeris* and *N. barkeri* Hughes (note that in addition *Lasioseius bispinosus* Evans was found on infested tulip bulbs in Niigata, Japan; Lesna, personal observation, 1992). Each of these predator species had the ability to increase their population size when fed exclusively with dry bulb mites, but *N. cucumeris* showed the highest capacity of population growth and managed to get access to interior of the bulb, albeit only in bulbs stored in closed cardboard boxes (a method rarely used in practice), where ethylene levels are higher and induce modifications in interior bulb

structure that promote predator access (Lesna et al. 2005). Since none of the other predators found in The Netherlands, so far, showed an ability to reach the bulb's inside under storage conditions in practice, the only solution was to find predators that feed on eriophyid mites and are small enough to cope with the narrow space inside a bulb. This is why the impetus for the work presented in this article came from the discovery of *N. paspalivorus*, a very small predatory mite feeding on eriophyid mites under the perianth of coconut fruits. Moreover, climatic conditions in bulb storage are not too different from those in coconut-growing areas of the world and dry bulb mites live in microhabitats that are structurally similar to those under the perianth of coconuts. For all these reasons we opted for this exotic predator instead of predators endemic in The Netherlands.

Perspectives for *Neoseiulus paspalivorus* in practice

To make biocontrol using this predator from coconuts available for application in practice there are still several hurdles to overcome, as summarized during a workshop in Lisse attended by representatives from all interested parties (Lommen et al. 2012c). The first is to test the use of biological control with *N. paspalivorus* at a larger scale and under practical conditions of bulb storage (e.g. more ventilation, decreasing temperatures over the course of the storage period). The second is to remove the predatory mites from the bulbs before export to countries with severe quarantine regulation. Last but not least there is a need to develop a method of mass rearing of *N. paspalivorus* on other prey or other foods, to bypass the expensive method of rearing them on eriophyid mites on host plants. Once these hurdles are taken, application in practice will be attractive to organic farmers and, if in the future the use of pesticides to treat bulbs becomes more restricted, it will be a major alternative for traditional farmers as well.

Our experiments with *N. paspalivorus* represent a clear-cut example of successful application of a predatory mite to control a different species of eriophyoid mite in microhabitats that are similar in structure but otherwise part of a completely different mircro- and macro-habitat. Hence, we consider this result as one that confirms the 'new' association hypothesis of Hokkanen and Pimentel (1989).

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References

Aratchige NS (2007) Biological control of rust mites in coconuts and tulip bulbs. PhD Thesis, University of Amsterdam

Aratchige NS, Lesna I, Sabelis MW (2004) Below-ground plant parts emit herbivore-induced volatiles: olfactory responses of a predatory mite to tulip bulbs infested by rust mites. Exp Appl Acarol 33:21–30

Asjes CJ, Blom-Barnhoorn GJ (1998) Verspreiding tulpenvirus X in tulpen—Nu meer bekend over de overbrenger. Bloembollencultuur 15:18–19

Conijn CGM, Van Aartrijk J, Lesna I (1996) Flower bulbs. In: Lindquist EE, Sabelis MW, Bruin J, Pests World Crop (eds) Eriophyoid mites. Their biology, natural enemies and control, 6th edn. Elsevier, Amsterdam, pp 651–659

de Kock MJD, Lommen STE, Lemmers MEC (2011) Voorkom vooral tulpengalmijt in bewaring: waakzaamheid voor TVX geboden. Bloembollenvisie 222:18–19

De Munk WJ (1973) Bud necrosis, a storage disease of tulips IV. The influence of ethylene concentration and storage temperature on bud development. Eur J Plant Pathol 79:13–22

Gude H, Dijkema M (2005) The use of 1-MCP as an inhibitor of ethylene action in tulip bulbs under laboratory and practical conditions. Acta Horticulturae 673:243–248

Hadfield J (2010) MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. J Stat Softw 33:1–22

Hokkanen HMT, Pimentel D (1989) New associations in biological control—theory and practice. Canadian Entomol 121:829–840

Kamerbeek GA, de Munk WJ (1976) A review of ethylene effects in bulbous plants. Sci Hortic 4:101–115

Lawson-Balagbo LM, Gondim MGC Jr, de Moraes GJ, Hanna R, Schausberger P (2008) Exploration of the acarine fauna on coconut palm in Brazil with emphasis on *Aceria guerreronis* (Acari: Eriophyidae) and its natural enemies. Bull Entomol Res 98:83–96

Lesna I, Conijn CGM, Sabelis MW (2005) From biological control to biological insight: rust-mite induced change in bulb morphology, a new mode of indirect plant defence? In: G. Weigmann, G. Alberti, A. Wohltmann and S. Ragusa (eds), Acarine Biodiversity in the Natural and Human Sphere. Phytophaga (Palermo) 14: 285–291

Lindquist EE, Sabelis MW, Bruin J (eds) (1996) Eriophyoid mites—their biology, natural enemies and control. World Crop Pest Series Vol. 6, Elsevier, Amsterdam, The Netherlands, 790 + xxxii pp

Lommen STE, Conijn CGM, Lemmers MEC, Pham KTK, de Kock MJD (2012a) Mites as vector of tulip virus X in stored tulip bulbs. Integrated protection of stored products. IOBC-WPRS Bulletin 81:57–67

Lommen S, Duyvesteijn R, Gude H (2012b) Kernrot in tulp: de risico's ontrafeld, de mythe ontmanteld. Bloembollenvisie 250:20–21

Lommen STE, Lesna I, Da Silva F, Sabelis M (2012c) Kleine roofmijt pakt tulpengalmijt effectief aan. Bloembollenvisie 260:22–23

Negloh K, Hanna R, Schausberger P (2011) The coconut mite, *Aceria guerreronis*, in Benin and Tanzania: occurrence, damage and associated acarine fauna. Exp Appl Acarol 55:361–374

R Core Team (2011) R: A language and environment for statistical computing. Vienna, Austria. http://www.R-project.org

Sabelis MW (1996) Phytoseiidae. In: Lindquist EE, Sabelis MW, Bruin J (eds) Eriophyoid mites—their biology, natural enemies and control. World Crop Pest Series, vol 6. Elsevier, Amsterdam, pp 427–456

Sabelis MW, Bruin J (1996) Evolutionary ecology: life history patterns, food plant choice and dispersal. In: Lindquist EE, Sabelis MW, Bruin J (eds) Eriophyoid mites—their biology, natural enemies and control. World Crop Pest Series, vol 6. Elsevier, Amsterdam, pp 329–366

Sabelis MW, Lesna I, Aratchige NS (2007) A tritrophic perspective to the biological control of eriophyoid mites. IOBC/WPRS Bulletin 30(5):91–93

Sabelis MW, Janssen A, Lesna I, Aratchige NS, Nomikou M, van Rijn PCJ (2008) Developments in the use of predatory mites for biological pest control. IOBC/WPRS Bulletin 12:187–199

Sabelis MW, Janssen A, Lesna I (2012) Perspective: consequences of trait-mediated indirect interactions for biological control of plant pests. In: Ohgushi T, Schmitz OJ, Holt RD (eds) Chapter 22 in: interaction richness and complexity: ecological and evolutionary aspects of trait-mediated indirect interactions. Cambridge University Press, Cambridge, pp 435–449

Van Aartrijk J (2000) Ziekten en afwijkingen bij Bolgewassen. Deel l: Liliaceae. Derde Druk. Laboratorium voor Bloembollenonderzoek, Lisse, p 194